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Part II

Department of Health and Human Services

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Recombinant DNA Research; Actions Under Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, HHS.

ACTION: Notice of actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH, under the 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules (45 FR 77384).

EFFECTIVE DATE: March 11, 1981.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205. (301) 496–6051.

SUPPLEMENTARY INFORMATION: I am promulgating today several major actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These proposed actions were published for comment in the **Federal Register** of November 28, 1980, and reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on January 8–9, 1981. In accordance with Section IV–E–1–b of the NIH Guidelines, I find that these actions comply with the Guidelines and present no significant risk to health or the environment.

Part I of this announcement provides background information on the actions. Part II provides a summary of the major actions.

I. Decisions on Actions Under Guidelines

I–A. Proposed Containment for Nonpathogens

Dr. Winston Brill, a RAC member, proposed in a letter dated November 14, 1980, that the Guidelines be amended to permit use of nonpathogenic prokaryotes and nonpathogenic lower eukaryotes as donors and recipients in recombinant DNA experiments under P1 containment conditions.

The following language was published in the November 28, 1980 **Federal Register** (45 FR 79386) for thirty days of comment:

A. A new section, III–O–2, would be added to the Guidelines, as follows:

"III–O–2. *Experiments Involving Non-Pathogenic Prokaryotes and Lower Eukaryotes.* Recombinant DNA experiments involving prokaryotes and lower eukaryotes, nonpathogenic [2A] for man, animals, or plants, can be conducted under P1 containment."

B. A new paragraph would be added just before Section III–O begins, as follows:

"When the reader finds that the containment level given for the same experiment is different in two different sections within Part III, he may choose which of the two levels he wishes to use for the experiment."

C. The second paragraph of Section III–B–3 would deleted. Revised Section III–B–3 would read as follows:

"III–B–3. *Non-HV1 Systems.* Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b))."

D. Section IV–E–1–b–2–(f) would be deleted. This Section currently reads as follows:

"IV–E–1–b–(2)–(f). Assigning containment levels for experiments in which both donor and recipient are non-pathogenic prokaryotes (see Section III–B–3)."

During the thirty day comment period eight letters supporting this proposal were received. Two additional letters, while supporting the proposal, recommended that containment be set at P2, rather than P1.

The RAC discussed the proposal at the January 8–9, 1981 meeting. Dr. Brill suggested that the proposed Section III–O–2 be modified in two ways: (1) following the word "plants" would be added the words, "and only DNA from such sources,"; and (2) text would be added specifying that the experiments must be reviewed with the local IBC with documentation that the organisms are not known pathogens. (Dr. Brill also initially recommended that containment for these experiments be raised from P1 to P2, but later said he was convinced by the arguments of some RAC members that P1 was appropriate).

During the ensuing discussion RAC members opposed to the motion pointed out that: (1) the proposal covers an enormous variety of organisms, (2) "nonpathogenic" may be defined differently by different IBCs and (3) no restriction is placed on the type of vectors to be used. A substitute motion was advanced by Dr. Gottesman which would provide for an expedited review procedure. Under this proposal, ORDA would review proposals involving nonpathogenic prokaryotes and lower eukaryotes on a case-by-case basis. The RAC, by a vote of six in favor, eight opposed, and two abstentions, denied this substitute motion.

The RAC continued discussion of Dr. Brill's motion. Some RAC members felt the proposal as written placed a burden of interpreting "pathogenicity" on IBCs. Nonetheless, sentiment supporting an expedited type of procedure for dealing with prokaryotic and lower eukaryotic nonpathogens was expressed. By a vote of nine in favor, eight opposed, and three abstentions, the RAC passed Dr. Brill's motion, as it had appeared in the **Federal Register**, with the two changes suggested by Dr. Brill at the meeting.

The substitute proposal which would permit ORDA to lower containment on proposals utilizing nonpathogenic prokaryotes and lower eukaryotes on a case-by-case basis, was again offered by Dr. Gottesman for consideration. The proposal was that, if Dr. Fredrickson should not accept Dr. Brill's proposal, it was the sense of the RAC that it would be preferable to the status quo to at least make two changes: (1) extend the current situation allowing cloning between nonpathogenic prokaryotes at P3 to also include lower eukaryotes; and (2) allow lowering below P3 for individual cases by ORDA rather than requiring RAC review. A motion to table this proposal failed; by a vote of eight in favor, eleven opposed. By a vote of fourteen in favor, one opposed, and three abstentions, the RAC then approved Dr. Gottesman's motion.

In deciding whether to accept the "Brill" motion, recommended by the RAC by a vote of nine in favor, eight opposed, and three abstentions, or the more conservative "Gottesman" motion, recommended by the RAC by a vote of fourteen in favor, one opposed, and three abstentions, I note that already in the Guidelines the following experiments are permitted:

1. Certain experiments with prokaryotes and lower eukaryotes are exempt from the Guidelines, under Sections I–E–3 and I–E–4.

2. Certain experiments with prokaryotes and lower eukaryotes may be done at P1 containment under Guideline Sections III–O, III–B–2 and III–C–5.

3. Certain experiments with prokaryotes and lower eukaryotes may be done at P2 containment under Guideline Sections III–A–1–a–(5) and III–A–1–b.

Dr. Brill's proposal covers an enormous number of organisms, many of which have not been well characterized. "Nonpathogenic" might be interpreted differently by different IBCs. The proposal does not require use of non-conjugative plasmids. For these reasons I am, at present, not accepting the "Brill" motion and instead I am accepting the more conservative "Gottesman" motion.

Accordingly a new section, III–O–2, is added to the Guidelines as follows:

"III–O–2. *Experiments Involving Prokaryotes Nonpathogenic for Man,*

Animals or Plants, and/or Lower Eukaryotes Nonpathogenic for Man, Animals or Plants.

Recombinant DNA experiments involving prokaryotes nonpathogenic for man, animals, or plants, and/or lower eukaryotes nonpathogenic for man, animals or plants, and only DNA from such sources, can be conducted under P3 containment (2A). Lower levels of physical containment may be assigned by ORDA on a case-by-case basis for specific donor-recipient combinations (see Section IV–E–1–b–(3)–(h))."

A new paragraph is added just before Section III–O begins, as follows:

"When the reader finds that the containment level given for the same experiment is different in two different sections within Part III, he may choose whichever of the two levels he wishes to use for the experiment."

The second paragraph of Section III–B–3 is deleted. Revised Section III–B–3 now reads as follows:

"III–B–3. *Non-HV1 Systems.* Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b))."

Section IV–E–1–b–(2)–(f) is deleted.

A new section IV–E–1–b–(3)–(h) is added as follows:

"IV–E–1–b–(3)–(h). Assigning containment levels for experiments in which both donor and recipient are nonpathogenic prokaryotes and/or nonpathogenic lower eukaryotes (see Section III–O–2)."

In order to clarify the relationship among Sections III–O, III–O–1, and III–O–2, the final sentence in the second paragraph under Part III is amended to read as follows:

"Prior IBC review is required for all other experiments described in the subsections of Part III, including III–O–1, III–O–2, etc."

I–B. Request for Approval of Schizosaccharomyces Pombe for Recombinant DNA Experiments

Dr. Benjamin D. Hall of the University of Washington, in letters dated November 3, 1980, and December 5, 1980, requested that the fission yeast, *Schizosaccharomyces pombe*, together with the *S. cerevisiae/E. coli* hybrid recombinant plasmids, be certified as an HV1 host-vector system. Dr. Hall further requested that this system be included under Section III–O of the Guidelines. In his submission, Dr. Hall noted that *Schizosaccharomyces pombe* is not a pathogen, it survives poorly outside of a controlled laboratory environment, it is rare in nature, and genetic exchange in the *Ascomycetes* occurs only with closely related organisms.

Dr. Hall's request was published in the November 28, 1980 **Federal Register** (45 FR 79387). No comments were received during the thirty day comment period.

The RAC, in discussing the proposal at the January 8–9, 1981 meeting, noted that specifications for HV1 certification have been defined in Section II–D–2–b of the Guidelines. It was suggested that only certain experiments be approved without certifying *S. pombe* as a general HV1 host-vector system. The RAC, therefore, recommended by a vote of fourteen in favor, one opposed and four abstentions, that DNA from non-pathogenic prokaryotes and lower eukaryotes may be cloned into *Schizosaccharomyces pombe* species under P1 containment conditions. It further recommended by a vote of fourteen in favor, none opposed and five abstentions, that DNA from higher eukaryotes may be cloned in *S. pombe* species under P3 containment conditions.

I accept these recommendations. Item 18 in Appendix E of the Guidelines is amended to read as follows:

"18. DNA from nonpathogenic prokaryotes and nonpathogenic lower eukaryotes may be cloned into *Schizosaccharomyces pombe* species under P1 containment conditions. DNA from higher eukaryotes may be cloned in *S. pombe* species under P3 containment conditions."

I–C. Request To Include Streptococcus Faecalis and Streptococcus Sanguis in Appendix A.

Dr. Donald Clewell of the University of Michigan requested in letters dated August 13, 1980 and September 10, 1980, that *Streptococcus faecalis* be included along with *Streptococcus sanguis* in a sublist of Appendix A. Recombinant DNA molecules composed entirely of DNA segments from organisms within each sublist of Appendix A are exempt from the Guidelines under Section I–E–4, which exempts "Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes. . . ." In support of his request, Dr. Clewell submitted data indicating that exchange of genetic information between the two species occurs by natural physiological processes.

A **Federal Register** announcement of Dr. Clewell's request appeared on November 28, 1980 (45 FR 79386). No comments were received during the thirty day comment period.

The RAC discussed Dr. Clewell's proposal at the January 8–9, 1981 meeting. The RAC was satisfied that the data demonstrated two-way plasmid transfer. By a vote of twelve in favor, two opposed and five abstentions, the RAC recommended that the request be approved, and that *Streptococcus faecalis* be added to sublist F of Appendix A.

I accept this recommendation, and Appendix A, Sublist F has been amended to read as follows:

"*Sublist F*

1. *Streptococcus sanguis*
2. *Streptococcus pneumoniae*
3. *Streptococcus faecalis*"

I–D. Request To Clone Saccharomyces Cerevisiae DNA in Tetrahymena Thermophilia

Dr. Eduardo Orias of the University of California, Santa Barbara in a letter dated December 18, 1980 requested permission to clone *Saccharomyces cerevisiae* DNA in *Tetrahymena thermophila* using *S. cerevisiae/E. coli* hybrid plasmids.

Dr. Orias supplied data outlining the genetics and ecology of *Tetrahymena*. He noted that *Tetrahymena thermophila* is a unicellular microbial eukaryote of no known pathogenicity. Dissemination of genetic information between members of the species by means other than eukaryotic conjugation has not been demonstrated.

Dr. Orias' request appeared in the November 28, 1980 **Federal Register** (45 FR 79387). No comments were received during the thirty day comment period.

During the RAC discussion of the proposal at the January 8–9, 1981 meeting, it was noted that the proposal involves nonpathogenic organisms, and approval at the P1 level of containment was recommended by a unanimous vote of nineteen in favor.

I accept this recommendation, and a new entry has been added to Appendix E to read as follows:

"26. *Saccharomyces cerevisiae* DNA may be cloned in *Tetrahymena thermophila* using *E. coli/S. cerevisiae* hybrid plasmids under P1 containment conditions."

I–E. Proposal To Amend Item 4 of Appendix E

Entry 4 of Appendix E of the Guidelines reads as follows:

"4. Cloned desired fragments from any non-prohibited source may be transferred into *Agrobacterium tumefaciens* containing a Ti plasmid (or derivatives thereof), using a nonconjugative *E. coli* plasmid vector

coupled to a fragment of the Ti plasmid and/or the origin of replication of an *Agrobacterium* plasmid, under containment conditions one step higher than would be required for the desired DNA in HV1 systems (i.e., one step higher physical containment than that specified in the subsections of Section III–A). Transfer into plant parts or cells in culture would be permitted at the same containment level (one step higher)."

Dr. Clarence Kado of the University of California, Davis, proposed in a letter dated October 2, 1980 that the fourth entry in Appendix E be modified to read as follows:

"4. Cloned desired fragments from any non-prohibited source may be transferred into *Agrobacterium tumefaciens* containing a Ti plasmid (or derivatives thereof), using a nonconjugative *E. coli* plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of an *Agrobacterium* plasmid, under containment conditions that would be required for the desired DNA in HV1 systems (i.e., that specified in the subsections of Section III–A). Transfer into plant parts or cells in culture would be permitted at the same containment level."

An announcement of Dr. Kado's proposed amendment was published in the **Federal Register** of November 28, 1980 (45 FR 79387). No comments were received during the thirty day comment period.

During the discussion of the proposal at the January 8–9, 1981 RAC meeting, it was noted that this proposal would effectively designate *Agrobacterium tumefaciens* an HV1 system.

It was pointed out that while *Agrobacterium tumefaciens* is a plant pathogen, plant tissue must be injured to permit infection. Further, the genetic information introduced into plant cells by the *Agrobacterium tumefaciens* Ti plasmid is not stably integrated into the plant genome and is not transmitted at meiosis into seeds. Reservations were expressed, however, about approving *A. tumefaciens* as an HV1 system. A motion to permit cloning of DNA from plants and nonpathogenic prokaryotes in *Agrobacterium tumefaciens* with subsequent transfer to plants or plant tissue under P2 containment conditions was recommended by a vote of seventeen in favor, none opposed and two abstentions.

I accept this recommendation, and entry 4 of Appendix E, is amended to read as follows:

"4. Cloned desired fragments from any non-prohibited source may be transferred into *Agrobacterium tumefaciens* containing a Ti plasmid (or derivatives thereof), using a nonconjugative *E. coli* plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of an *Agrobacterium* plasmid, under containment conditions one step higher than would be required for the desired DNA in HV1 systems (i.e., one step higher than that specified in the subsections of Section III–A). However, DNA from plants and nonpathogenic prokaryotes may be cloned under P2 containment conditions. Transfer into plant parts or cells in culture is permitted at the same containment level as is used for the cloning in *Agrobacterium tumefaciens.*

I–F. Request for Lowering of Containment Under Entry Four of Appendix E

Dr. Mary-Dell Chilton of Washington University in St. Louis, in a letter of September 10, 1980, requested a reduction in physical containment, to the P2 level, for the manipulation in *Agrobacterium tumefaciens* of (1) the *Saccharomyces cerevisiae* alcohol dehydrogenase 1 gene and (2) the gene coding for the maize (*Zea mays*) seed storage protein, zein. The cloned DNA and the cloning vectors will be introduced into tobacco plants. These experiments are currently covered under entry 4 of Appendix E which stipulates use of P3 containment. Dr. Chilton's proposal was published for comment in the November 28, 1980 **Federal Register** (45 FR 79387). No comments were received during the thirty day comment period.

Noting that the cloned DNA sequences to be manipulated are well characterized, the RAC at the January 8–9, 1981 meeting recommended, by a vote of fifteen in favor, none opposed and four abstentions, P1 containment conditions for the described experiments.

I accept this recommendation, and text has been added to entry 4 of Appendix E, so indicating.

I–G. Proposed Procedures for Previously Approved Large-Scale Recombinant DNA Experiments

1. *Application Procedures for Minor Modifications of Previously Approved Large-Scale Recombinant DNA Experiments.* The following procedures for handling minor modifications of previously approved large-scale recombinant DNA experiments, generated by a Working Group of the Recombinant DNA Advisory Committee (RAC), were published in the November 28, 1980 **Federal Register** (45 FR 79387):

"Procedures have been developed for considering applications to grow more than ten liters of an organism containing recombinant DNA. These procedures include consideration of the request by a working group of the NIH Recombinant DNA Advisory Committee, submission of the request to the full RAC after consideration by the working group, and subsequent submission to the Director, NIH, for final review. This procedure has taken a minimum of two months. Therefore, the following procedures are proposed to expedite consideration of requests to grow more than ten liters of recombinant DNA-containing organisms when these proposals represent minor modifications of previously approved experiments. Modifications include deletion of sequences from the recombinant DNA, changes in promoters, addition of short seqments not affecting the nature of the expressed products, and minor changes in the properties of the host. Changes are considered minor if they do not affect either the containment properties of the vector or the host, or the nature of products made, or add new products. Therefore, the procedures for dealing with minor modifications have the objective of determining that the change is indeed minor. To determine whether a change is minor, two levels of review will take place:

"(1) By ORDA, which will decide upon receipt of a request to process it as a new request or as a minor modification, and in the latter case

"(2) by a working group of at least two members of RAC.

"The Working Group has proposed that the following language be added to the 'Application Procedures for Large-Scale Recombinant DNA Experiments':

"6. Proposals that the submitter considers to represent minor modifications of already approved experiments will be handled by an expedited procedure. A request must be submitted to ORDA. This request should include the changes made, the way in which these changes were made (e.g., mutagenesis, recloning), and the nature and results of any tests done to determine that no major change has occurred (e.g., restriction enzyme analysis, tests for produced products, tests of vector mobilization).

"ORDA will determine whether the submission represents a minor modification of an approved experiment. If so, the request will be submitted promptly to a working group of at least two RAC members. If possible, these members should have been present at the RAC discussion of approval of the original experiment. If any member of

the working group does not agree that the request represents a minor modification, the application will then be referred to the full RAC at its next meeting. If the working group is unanimous in concluding that the changes do not alter the organism in a way that is likely to affect containment of the organism or the vector, or the nature of the expressed product, significantly, from that presented originally to RAC, recommendation for approval will be transmitted to ORDA, and, through ORDA, to the submitters. Consideration of requests by working groups should explicitly address the following issues: Is the change likely to compromise biological containment provided by the host or the vector? Does the change add to the biological activities associated with the expressed products in a way not considered by the original submission?"

During the thirty day comment period, no comments were received.

Prior to publication in the **Federal Register**, the RAC discussed the proposal at the September 25–26, 1980 meeting; the proposed procedures were considered to be reasonable.

The RAC subsequently discussed the proposal at the January 8–9, 1981 meeting and by a vote of seventeen in favor, none opposed and one abstention, recommended approval of the proposed language.

I accept this recommendation. The complete text of the revised procedures for review of large-scale experiments appears in Part II of this announcement.

2. *Proposed Procedures for Change of Locale of Previously Approved Large-Scale Recombinant DNA Experiments.* During the discussion of proposed procedures dealing with minor modifications of previously approved large-scale recombinant DNA experiments at the January 8–9, 1981, RAC meeting, the question of how to process changes of site for large-scale production was raised. In this situation, recombinant clones, which had been approved for scale-up at one site, would be moved to another physical facility. A second IBC might then be charged with oversight responsibilities.

RAC recommended that a registration document, which would indicate compliance with the NIH Guidelines and its large-scale procedures should be filed with ORDA by the IBC of the new institution. ORDA could then give approval for large-scale growth of the clones at the new site. No RAC review would be required.

I accept this recommendation. Accordingly, the following additional language will be added to the "Application Procedures for Large-Scale Recombinant DNA Experiments":

"7. Should a clone, previously approved for scale-up at one physical facility, be proposed to be moved to a second physical facility, the IBC with oversight responsibility at the second site shall submit to ORDA a registration document and receive ORDA approval, prior to initiating scale-up. No RAC review would be required.

The complete text of the revised procedures for review of large-scale experiments appears in Part II of this announcement.

I–H. Proposals To Clone Genes of Foot and Mouth Disease Virus

The RAC at its December 6–7, 1979, meeting had reviewed a four stage proposal submitted by Dr. Howard Bachrach of the United States Department of Agriculture Plum Island Animal Disease Center and Dr. Dennis Kleid of Genentech, Inc., entitled "Cloning and Expression in *E. coli* of the VP3 protein of Foot and Mouth Disease Virus." In the **Federal Register** of January 17, 1980 (45 FR 3552), I, as Director, NIH, accepted the RAC recommendation to allow Stage I to proceed. At that time I noted, "Dr. Campbell stated that it was the sense of the RAC that this motion constituted the 'major action' and that future recommendations of the RAC approving further stages of the experiment would be 'minor actions.'"

In the **Federal Register** of July 29, 1980 (45 FR 50528), I, as Director, NIH, accepted a recommendation made by the RAC at its June 5–6, 1980, meeting that certain clones containing cDNA copies of pieces of the Foot and Mouth Disease virus, made on Plum Island under Stage I of the protocol, be allowed to be removed from Plum Island, as they "were well characterized, lacked infectivity, and represent, in aggregate, only 75% of the FMD viral genome."

On January 9, 1981, the RAC reviewed a request of October 17, 1980, from Dr. Kleid to modify Stage IV of the protocol, dealing with cloning of various FMD types. The RAC by a vote of 20 in favor, none opposed, and one abstention, recommended that this be accepted with the conditions that a working group of the RAC, but not the full RAC, would examine data on the infectivity of the clones produced on Plum Island, before they were allowed to leave Plum Island, and that such clones be well characterized, shown to lack infectivity, and shall not contain, individually or collectively, more than 75% of the viral genome.

I accept this recommendation.

I–J. Containment Levels for Recombinant DNA Experiments Involving Streptomyces and Other Non-Pathogenic Actinomycetes

Dr. Stanley Cohen of Stanford University Medical Center, in a letter dated November 18, 1980, submitted the following proposal for consideration by the RAC:

"That all members of the non-pathogenic *Actinomycetes* genus *Streptomyces* and the plasmids native to this genus be approved as host-vector systems for the cloning under P1 conditions of DNA derived from other non-pathogenic prokaryotic organisms such as *Streptomyces* and other non-pathogenic *Actinomycetes* species, *Escherichia coli* K–12, *Bacillus subtilis*, *Bacillus lichenformis*, *Bacillus circulans*, and other non-pathogenic *Bacillus* species, and for the cloning of DNA derived from non-pathogenic unicellular eukaryotic micro-organisms such as *Saccharomyces cerevisiae* and *Neurospora crassa*."

The RAC noted that they had recommended earlier in the meeting (see I–A above) that all experiments involving nonpathogenic prokaryotes and lower eukaryotes, be allowed at P1. Dr. Cohen's request involves a subset of these. The RAC, by a vote of 18 in favor, none opposed, and two abstentions, recommended that the proposal be accepted.

I accept this recommendation, and a new entry will be added to Appendix E as follows:

"27. All members of the nonpathogenic *Actinomycetes* genus *Streptomyces* and the plasmids native to this genus are approved as host-vector systems for the cloning under P1 conditions of DNA derived from other nonpathogenic prokaryotic organisms such as *Streptomyces* and other nonpathogenic *Actinomycetes* species, *Escherichia coli* K–12, *Bacillus subtilis*, *Bacillus lichenformis*, *Bacillus circulans*, and other nonpathogenic *Bacillus* species, and for the cloning of DNA derived from nonpathogenic unicellular eukaryotic microorganisms such as *Saccharomyces cerevisiae* and *Neurospora crassa*."

II. Summary of Actions Under Guidelines—Arranged According to Position in Guidelines

II–A. Amendment of Second Paragraph of Part III

The final sentence in the second paragraph under Part III is amended to read as follows:

"Prior IBC review is required for all other experiments described in the

subsections of Part III, including III–O–1, III–O–2, etc."

II–B. Addition of New Paragraph to Part III

A new paragraph is added just before Section III–O begins, as follows:

"When the reader finds that the containment level given for the same experiment is different in two different sections within Part III, he may choose whichever of the two levels he wishes to use for the experiment."

II–C. Addition of New Section III–O–2

A new section, III–O–2, is added to the guidelines as follows:

"III–O–2. *Experiments Involving Prokaryotes Nonpathogenic for Man, Animals or Plants, and/or Lower Eukaryotes Nonpathogenic for Man, Animals or Plants.* Recombinant DNA experiments involving prokaryotes nonpathogenic for man, animals, or plants, and/or lower eukaryotes nonpathogenic for man, animals or plants, and only DNA from such sources, can be conducted under P3 containment (2A). Lower levels of physical containment may be assigned by ORDA on a case-by-case basis for specific donor-recipient combinations (see Section IV–E–1–b–(3)–(h))."

II–D. Amendment of Section III–B–3

The second paragraph of Section III–B–3 is deleted. Revised Section III–B–3, now reads as follows:

"III–B–3. *Non-HVI Systems.* Containment levels for other classes of experiments involving non-HVL systems may be approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), IV–E–1–b–(3)–(b))."

II–E. Deletion of Section IV–E–1–b–(2)–(f)

Section IV–E–1–b–(2)–(f) is deleted.

II–F. Addition of Section IV–E–1–b–(3)–(h)

A new section IV–E–1–b–(3)–(h) is added as follows:

"IV–E–1–b–(3)–(h). Assigning containment levels for experiments in which both donor and recipient are nonpathogenic prokaryotes and/or nonpathogenic lower eukaryotes (see Section III–O–2)."

II–G. Amendment of Appendix A, Sublist F

Appendix A, Sublist F is amended to read as follows:

"Sublist F

1. *Streptococcus sanguis*
2. *Streptococcus pneumoniae*
3. *Streptococcus faecalis"*

II–H. Amendments of Appendix E

Entires 4 and 18 of Appendix E, are amended to read as follows:

"4. Cloned desired fragments from any non-prohibited source may be transferred into *Agrobacterium tumefaciens* containing a Ti plasmid (or derivatives thereof), using a nonconjugative *E. coli* plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of an *Agrobacterium* plasmid, under containment conditions one step higher than would be required for the desired DNA in HVl systems (i.e., one step higher than that specified in the subsections of Section III–A). However, DNA from plants and nonpathogenic prokaryotes may be cloned under P2 containment conditions; and the *Saccharomyces cerevisiae* alcohol dehydrogenase 1 gene and the gene coding for the maize (*Zea mays*) seed storage protein, zein, may be cloned under P1 conditions. Transfer into plant parts or cells in culture is permitted at the same containment level as is used for the cloning in *Agrobacterium tumefaciens.*

"18. DNA from nonpathogenic prokaryotes and nonpathogenic lower eukaryotes may be cloned into *Schizosaccharomyces pombe* species under P1 containment conditions. DNA from higher eukaryotes may be cloned in *S. pombe* species under P3 containment conditions."

The following new entries are added to Appendix E:

"26. *Saccharomyces cerevisiae* DNA may be cloned in *Tetrahymena thermophila* using *E. coli/S. cerevisiae* hybrid plasmids under P1 containment conditions."

"27. All members of the nonpathogenic *Actinomycetes* genus *Streptomyces* and the plasmids native to this genus are approved as host-vector systems for the cloning under P1 conditions of DNA derived from other nonpathogenic prokaryotic organisms such as *Streptomyces* and other nonpathogenic *Actinomycetes* species, *Escherichia coli* K–12, *Bacillus subtilis, Bacillus lichenformis, Bacillus circulans,* and other nonpathogenic *Bacillus* species, and for the cloning of DNA derived from nonpathogenic unicellular eukaryotic microorganisms such as *Saccharomyces cerevisiae* and *Neurospora crassa."*

II–I. Procedures for Review of Large-Scale Experiments

The following procedures, which are not incorporated into the Guidelines, have been adopted for the review of large-scale experiments. (They are an expansion of the procedures which appeared in the November 21, 1980 **Federal Register** (45 FR 77380)):

"Application Procedures for Large-Scale Recombinant DNA Experiments.

"1. For each research project proposing to exceed the 10-liter limit, the applicant shall file a request with the NIH Office of Recombinant DNA Activities (ORDA). The request should include the following information:

"a. The registration document submitted to the local Institutional Biosafety Committee (see Section III of the Guidelines). This should include, or have appended to it, a summary paragraph which describes the proposed project in language that is comprehensible to non-specialists.

"b. A statement of the rationale for wishing to exceed the 10-liter limit.

"c. Evidence that the recombinant DNAs to be employed in the research have been rigorously characterized and are free of harmful sequences.

"d. Specification of the P–LS level proposed to be used as defined in the NIH Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules (**Federal Register,** April 11, 1980).

"2. Each request submitted to ORDA shall be referred to a working group of the NIH Recombinant DNA Advisory Committee for review.

"3. Following review and approval by the working group, each request shall be submitted to the entire Recombinant DNA Advisory Committee for review.

"4. Following review and approval by the RAC, each request shall be submitted to the Director, NIH, for the final review.

"5. Applications for large-scale experiments which are submitted by institutions not receiving NIH funds for recombinant DNA research shall be kept confidential (provided the institutions so desire) in accordance with the provisions of the NIH Guidelines for Research Involving Recombinant DNA Molecules and to the extent permitted by law.

"6. Proposals that the submitter considers to represent minor modifications of already approved experiments will be handled by an expedited procedure. A request must be submitted to ORDA. This request should include the changes made, the way in which these changes were made (e.g., mutagenesis, recloning), and the nature and results of any tests done to determine that no major change has occurred (e.g., restriction enzyme analysis, tests for produced products, tests of vector mobilization). ORDA will

determine whether the submission represents a minor modification of an approved experiment. If so, the request will be submitted promptly to a working group of at least two RAC members. If possible, these members should have been present at the RAC discussion of approval of the original experiment. If any member of the working group does not agree that the request represents a minor modification, the application will then be referred to the full RAC at its next meeting. If the working group is unanimous in concluding that the changes do not alter the organism in a way that is likely to affect containment of the organism or the vector, or the nature of the expressed product significantly from that presented originally to RAC, recommendation for approval will be transmitted to ORDA, and, through ORDA, to the submitters. Consideration of requests by working groups should explicitly address the following issues: Is the change likely to compromise biological containment provided by the host or the vector? Does the change add to the biological activities associated with the expressed products in a way not considered by the original submission?

"7. Should a clone, previously approved for scale-up at one facility, be proposed to be moved to a second facility, the IBC with oversight responsibility at the second site shall submit to ORDA a registration document and receive ORDA approval, prior to initiating scale-up. No RAC review would be required.

"8. These procedures may be refined or revised on the basis of discussion and action by the NIH Recombinant DNA Advisory Committee."

Additional Announcements of the Director, NIH

Section IV–E–1–b–(3)–(d) of the Guidelines gives responsibility to the Director, NIH, for "authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences."

Accordingly, several requests for authorization to culture, on a large-scale, recombinant DNA host-vector systems have been received and reviewed by the NIH.

I. Genentech, Inc.

On February 2, 1981, the Director, NIH, on the recommendation of the RAC, approved a request from Genentech, Inc., for the large-scale culture of EK1 host-vector systems containing plasmids coding for human leukocyte interferons.

This request was approved with the understanding that Genentech, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site.

The principal investigator is Dr. Norm S. C. Lin. The work is to be done at the P1–LS level of containment at the research and development facility at 460 Point San Bruno Boulevard, South San Francisco, California 94080.

II. Genentech, Inc.

On February 2, 1981, the Director, NIH, on the recommendation of the RAC, approved a request from Genentech, Inc., for the large-scale culture of EK1 host-vector systems containing plasmids coding for bovine growth hormone.

This request was approved with the understanding that Genentech, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities should NIH choose to inspect the site.

The principal investigator is Dr. Norm S. C. Lin. The work is to be done at the P1–LS level of containment at the research facility at 460 Point San Bruno Boulevard, South San Francisco, California 94080.

III. Hoffman-LaRoche, Inc.

On February 2, 1981, the Director, NIH, on the recommendation of the RAC, approved a request from Hoffman-LaRoche, Inc., for the large-scale culture of EK1 host-vector systems containing plasmids coding for human leukocyte and fibroblast interferons.

This request was approved with the understanding that Hoffman-LaRoche, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site.

The principal investigators are Drs. Philip A. Miller and Vishra Rai. The work is to be done at the P1–LS level of containment at the research and development facilities at 340 Kingsland Avenue, Nutley, New Jersey 07110.

IV. Burns-Biotec Laboratories, Inc.

On February 20, 1981, the Director, NIH, on the recommendation of the RAC, approved a request from Burns-Biotec, Inc., a wholly owned subsidiary of Schering-Plough Corporation, for the large-scale culture of EK1 host-vector systems containing plasmids coding for human leukocyte interferon.

This request was approved with the understanding that Burns-Biotec, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site.

The principal investigator is Dr. Donald Baldwin. The work is to be performed at the P1–LS level of containment at the facilities in Elkhorn, Indiana 68022.

V. Schering-Plough Corporation

On February 20, 1981, the Director, NIH, on the recommendation of the RAC, approved a request from Schering-Plough Corporation for the large-scale culture of EK1 host-vector systems containing plasmids coding for human leukocyte interferon.

This request was approved with the understanding that Schering-Plough Corporation has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site.

The principal investigator is Dr. Paul J. Leibowitz. The work is to be performed at the P1–LS level of containment at the research and development facilities in Bloomfield, New Jersey 07003.

Dated: March 4, 1981.

Donald S. Fredrickson,

Director, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.* Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8–(b)–(4) and (5) of that Circular.

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